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SLEEPING SICKNESS BUREAU
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Experiments to ascertain if Cattle may act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

BY COLONEL SIR DAVID BRUCE, C.B., F.R.S., A.M.S.; CAPTAINS A. E. HAMERTON, D.S.O., AND H. R. BATEMAN, R.A.M.C.; AND CAPTAIN F. P. MACKIE, I.M.S.

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Experiments to ascertain if Cattle may act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908—10.)

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The question as to whether cattle can act as a reservoir of the virus of Sleeping Sickness is an important one. It was usually believed until lately that man was the main reservoir, and that the other animals might be ignored. But in view of the fact that the fly on the Lake-shore have remained infective for some two years after the native population have been removed, it is necessary to enquire if it is not possible that other animals may act as well.

In this regard cattle have been, perhaps, the most important, as on the once thickly-populated Lake-shore and islands they were numerous, and in many cases grazed and watered in the fly-area. Another reason of their importance is, that if they can act as a reservoir, then the same will probably be true of the different species of antelope which inhabit the Lake-shore. It may be presumed that these will greatly increase now that the natives and domestic animals have been removed, and that they will take the place of the cattle in keeping up the infectivity of the *Glossina palpalis*.

The Commission, therefore, thought it would be well to enquire into the question, and the result is given in this paper.

Answers to the following questions were sought: Are cattle capable of being infected with Sleeping Sickness by the sub-cutaneous injection of blood containing *Trypanosoma gambiense?* Can cattle be infected with Sleeping Sickness by the bites of artificially-infected tsetse flies? Can cattle be infected with Sleeping Sickness by the bites of the naturally-infected flies caught on the Lake-shore? Is it possible to infect tsetse flies by feeding them on cattle infected with Sleeping Sickness, and afterwards to transmit the disease by means of these flies to healthy animals? Finally, if these questions are answered in the affirmative, will it be possible to find that cattle which have lived in the fly-area are naturally infected with Sleeping Sickness?

I. *Are Cattle capable of being Infected with Sleeping Sickness by the Subcutaneous Injection of Blood containing* Trypanosoma gambiense ?

Experiment 869. Bull.

September 10, 1909.—A bull was inoculated with 5 c.c. of blood containing large numbers of *Trypanosoma gambiense* from an infected monkey.

Its blood was examined daily, and 18 days after injection the bull was found to be infected with *Trypanosoma gambiense.* The identity of the trypanosome was established by injecting a monkey with some blood from the ox. This monkey showed *Trypanosoma gambiense* on the sixth day.

Conclusion.—From this experiment it is seen that oxen are capable of being infected with Sleeping Sickness by the injection of blood containing *Trypanosoma gambiense.* The trypanosome appears in small numbers in the blood, and the blood, when injected into susceptible animals such as monkeys, gives rise to a fatal form of the disease.

II. *Can Cattle be Infected with Sleeping Sickness by the Bites of Artificially-infected* Glossina palpalis ?

The two following experiments were carried out by feeding *Glossina palpalis* first on an infected monkey and immediately afterwards on a healthy ox. Wild flies from the Lake-shore were used.

Experiment 890. Ox.

May 20, 1909.—The ox was thrown and a monkey heavily infected with Sleeping Sickness was laid across its flank. Two cages of *Glossina palpalis*, containing 100 and 150 flies respectively, were allowed to feed for a few seconds on the monkey and then on the ox. The flies were allowed from 30 to 35 interrupted feeds on each animal every day. This was continued for 38 days, during which time 561 flies were estimated to have fed on one or other animal.

July 17.—Fifty-eight days after the first infected feed, *Trypanosoma gambiense* appeared in the blood of the ox.

The identity of the trypanosome was established by injection of the ox's blood into two monkeys. The first monkey was injected with blood from the ox 76 days and the second monkey 181 days after the flies had first fed on the ox. Both monkeys developed Sleeping Sickness, the first 7 days and the second 11 days after injection of the blood.

Experiment 891. Calf.

The details of this experiment were similar to those of the last. *Trypanosoma gambiense* appeared in the blood of the calf 57 days after the flies had been first fed upon it.

Three cubic centimetres of the blood of the calf were injected into a monkey, and the monkey developed Sleeping Sickness after an incubation period of 8 days.

Conclusion.—These two experiments show that when artificially infected *Glossina palpalis* are allowed to feed on healthy cattle, these animals develop Sleeping Sickness and that the blood of the cattle is capable of giving rise to infection of *Trypanosoma gambiense* in monkeys when injected into them.

III. *Can Cattle be Infected with Sleeping Sickness by the Bites of the Naturally-infected Flies caught on the Lake-shore?*

In the next three experiments freshly-caught *Glossina palpalis* brought up to the laboratory from the Lake-shore were allowed to feed straightway on healthy cattle. By this means it will be shown whether *Glossina palpalis* in their wild state are capable of giving Sleeping Sickness to healthy cattle.

Experiment 982. Bull.

2195 freshly captured *Glossina palpalis* were applied to a bull, and of these 1536 were estimated to have fed. This feeding of the flies extended over a period of 16 days, at the end of which time *Trypanosoma gambiense* appeared in the blood of the bull.

To help in the identification of this trypanosome, 3 c.c. of the blood of the bull were injected into a monkey. The monkey developed Sleeping Sickness 18 days later. 5 c.c. of the blood of the bull were also injected into a goat. *Trypanosoma gambiense* appeared in the blood of the goat after an incubation period of 38 days.

Experiment 1462. Bull.

The details of this experiment were similar to those of the last one. Over a period of eight days, 1370 wild flies from the Lake-shore were applied to the bull, of which 705 fed. Ten days from the first application of flies, *Trypanosoma gambiense* appeared in the blood of the bull.

Two animals, a monkey and a goat, each received 1 c.c. of the blood of the bull by injection under their skin. The monkey developed Sleeping Sickness seven days later, but the goat died in 16 days without showing any infection.

Experiment 1465. Bull.

During a period of 13 days, 459 freshly caught Lake-shore *Glossina palpalis* were applied to a bull, and of these 314 fed. On the fourteenth day after the flies were first fed, the bull developed an infection of *Trypanosoma gambiense*.

Some blood from this bull was injected into a monkey and into a goat. Neither animal became infected.

Conclusion.—These experiments prove that *Glossina palpalis*, when captured in their natural state on the Lake-shore, are capable of transmitting the virus of Sleeping Sickness to cattle, and that the blood of these cattle gives rise to a fatal form of the disease in monkeys and in goats when it is injected into them.

IV. *Is it possible to Infect Tsetse Flies by Feeding them on Cattle Infected with Sleeping Sickness, and afterwards to transmit the Disease by means of these Flies to Healthy Animals?*

Five experiments under this heading were carried out. Laboratory-bred flies were used in all of them. Three were negative and two positive. The three negative experiments will be shortly summarised first.

Experiment 1451.

Ninety laboratory-bred *Glossina palpalis* were fed for 10 successive days on a calf whose blood contained *Trypanosoma gambiense*. The flies were starved for 72 hours. They were then fed on a clean monkey daily for 45 successive days. The monkey failed to develop Sleeping Sickness.

When the remainder of the flies were dissected, one contained flagellates, but when the contents of this fly were injected into a goat the animal failed to show any infection of *Trypanosoma gambiense*.

Result.—Negative.

Experiment 1269.

The details of this experiment were similar to those of the last. After the *Glossina palpalis* had been fed on two oxen whose blood contained *Trypanosoma gambiense*, they were applied daily to a monkey. They were fed on this monkey for 35 consecutive days and were then transferred to a second monkey. Both the monkeys remained healthy.

Two of the flies were found on dissection to contain flagellates, but when these were injected into a monkey and a goat no development of Sleeping Sickness took place in these animals.

Result.—Negative.

Experiment 1672.

Here again the technique was similar to the last. The *Glossina palpalis* were fed, on alternate days for a lengthened period, on a clean monkey and a clean goat. Both animals remained healthy.

Some infected flies were found on dissection, but when introduced under the skin of a goat and of a monkey did not give rise to Sleeping Sickness.

Result.—Negative.

The next two experiments, which were carried out in the same way as the two preceding ones, were positive.

Experiment 1566.

The *Glossina palpalis* were fed on an infected ox, and after a starve of 72 hours were fed on a clean monkey for 45 successive days. 68 days after the flies had taken their first infected feed, this monkey developed Sleeping Sickness.

When the flies came to be dissected, nine of them showed flagellates either in the proboscis or in the alimentary tract. Some of these were injected into goats and into a monkey, but with negative results.

Result.—Positive.

Experiment 1602.

Fifty laboratory-bred flies were fed for four successive days on an ox whose blood contained *Trypanosoma gambiense*. After a period of starvation they were applied to a monkey and to a goat on alternate days. The monkey died before it could have become infected, but the goat developed Sleeping Sickness 20 days after the flies had their first infected feed.

The remainder of the flies, 32 in all, were dissected, and five were found to contain

flagellates. The alimentary contents of one of these flies were injected into a monkey, and after an incubation period of 13 days *Trypanosoma gambiense* appeared in its blood.

Result.—Positive.

Conclusion.—Laboratory-bred tsetse flies can be infected by feeding them on cattle infected with Sleeping Sickness, and afterwards the disease can be transmitted to healthy animals by means of these flies.

V. *Do Cattle, when Living in the Fly-area, actually carry the Virus of Sleeping Sickness?*

About seventeen cattle from various sources were examined with this point in view. Not all these cattle could be proved to have been exposed to the bites of *Glossina palpalis*, but most of them came from places where these flies are plentiful. One was positive.

Experiment 1633.

This cow came from the island of Kome, in Lake Victoria, where human Sleeping Sickness is prevalent and where *Glossina palpalis* abound.

Trypanosoma gambiense was found in its blood by microscopical examination, and when 3 c.c. of the blood were injected under the skin of a monkey the animal developed Sleeping Sickness after an incubation period of seven days.

Conclusion.—This experiment proves that cattle in their natural state, and apparently in good health, may harbour the virus of Sleeping Sickness.

General Conclusions.

It has been proved by experiment that cattle may act as a reservoir of the virus of Sleeping Sickness, and that healthy animals may be infected from them by means of *Glossina palpalis.*

It has also been proved that cattle in the fly-area do naturally harbour *Trypanosoma gambiense.*

It is, therefore, possible that the cattle and antelope living in the fly-area may act as a reservoir, and so keep up the infectivity of the *Glossina palpalis* for an indefinite period, but there is no proof up to the present that this actually takes place in Nature.

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